REMARKS

Claims 1-6, 20-24, 32 and 38-49 are presently pending. By this amendment, claims 1, 20 and 32 have been amended and new claims 43-49 have been added. It is believed that these amendments do not constitute new matter and their entry is requested. The Examiner has objected to the drawings, and formal drawings are being submitted concurrently with this response.

Objection to the Claims

The Examiner has objected to claim 32 for depending from claim 20 and reciting a method, while claim 20 recites a nucleic acid molecule. Claim 32 has been amended to recite the nucleic acid molecule of claim 20.

35 U.S.C. 112, First Paragraph Rejections

Claims 1-6 and 38-42 stand rejected under 35 U.S.C. 112, first paragraph, for lack of enablement. The claims were previously amended to recite methods for deleting DNA from an animal cell, yet the Examiner has maintained the position that, while the specification is enabling for a method for deleting a nucleic acid sequence in a specified cell or tissue in a mouse, the specification does not "enable" such methods for other organisms. It appears that the essence of these rejections is the Examiner's opinion that the scope of the claims encompasses animals and/or embryonic stem cells which are not themselves enabled.

In response, Applicants respectfully submit that, in rejecting certain of the claims, the Examiner has misinterpreted the scope of the presently pending claims and or has misapplied applicable U.S. patent law. The claims, as amended previously, encompass removal of DNA from an animal cell after said DNA has been inserted into the cell. The specification describes a working example wherein DNA is removed from an animal cell after it has been introduced into a cell. Nevertheless, in again rejecting the claims, the Examiner asserts that "certain embodiments of the invention require the use of a transgenic animal (Paper No. 14 at Page 5). The Examiner also is of the opinion that"ES cells are elements essential to **certain embodiments** of the invention" (*Id. emphasis added*). The Examiner is also of the opinion that certain

embodiments of the claims "encompass germ-line modification." (*Id.*) The Examiner is thus of the opinion that since certain claimed combinations, *e.g.*, ES cells from other than a mouse, are unpredictable, the claims are not enabled.

The fact that some of the claimed combinations may be inoperative does not necessarily jeopardize enablement. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 11569 (Fed. Cir. 1984). In *Atlas Powder*, the patent disclosed numerous salts, fuels, and emulsifiers that could form thousands of emulsions but there was no commensurate teaching as to which combination would work. The district court held that it would have been impossible for the inventor to list all operable emulsions and exclude the inoperable ones. *Id.* The Federal Circuit agreed: "Even if some of the claimed combinations were inoperative, the claims are not necessarily invalid. 'It is not a function of the claims to specifically exclude . . . possible inoperative substances . . .'" *Id.*, *citing*, In re Dinh-Nguyen, 492 F.2d 856, 858-59 (C.C.P.A. 1973). *In re Dinh-Nguyen*, 492 F.2d 856, 181 USPQ 46, which also holds that it is not a function of the claims to specifically exclude inoperative substances.

In rejecting the present claims, the Examiner appears to be of the opinion that certain specific claimed combinations are inoperative. As discussed below, Applicants do not agree that the Examiner has adequately provided evidence of any claimed combinations that are inoperable. Nevertheless, even assuming, *arguendo*, that certain claimed combinations are inoperable, based on applicable Federal Circuit case law, a rejection for lack of enablement for not excluding inoperative combinations is improper.

The enablement rejection of claims 1-6 and 38-42 is also improper for a separate but somewhat related reason. The "examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention." MPEP2164.04, *citing In re Wright*, 999 F.2d 1557, 1562, 27 U.S.P.Q.2d 1510, 1513 (Fed.Cir.1993). Furthermore, it is incumbent upon the Patent Office to

> "explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure."

MPEP § 2164.04, *citing In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971).

The Examiner has asserted that the claims are not enabled, emphasizing that there is a high degree of unpredictability associated with **generating** ES cells, **generating** transgenic animals and **effecting** germline gene therapy. The alleged nature of this unpredictability is thus cited for the "quantity of experimentation necessary to determine the parameters for achieving a method for **deleting** a nucleic acid sequence in the specified tissue of an organism." (Paper No. 14 at page 6) It is respectfully submitted that the Examiner has improperly cited technical reasons related to the generation of transgenic sequences in animals or cells as evidence of the unpredictability of deletion of such sequences. Such contentions, without scientific reasons or evidence are not sufficient to sustain an enablement rejection. As provided in the MPEP, if doubt arises about enablement because information is missing about one or more essential parts or relationships between parts which one skilled in the art could not develop without undue experimentation, the Examiner "should specifically identify what information is missing and why one skilled in the art could not supply the information without undue experimentation." MPEP, 2164.04. Furthermore, while references may not be required for the Examiner to meet his or her burden, "specific technical reasons are **always** required. Id emphasis added. Furthermore, to determine enablement, the specification is considered in light of the knowledge in the art at the time of the invention.

Regardless of whether the claimed nucleic acids can or cannot be introduced into a given cell or animal, the Examiner has provided no specific technical reasons for why the claimed methods will not work in any animal or cell following successful introduction of the claimed nucleic acids into said animal or cell.

The Examiner continues to apparently assert that germ-line gene therapy, i.e. integration and expression of a gene into the germline such that it is inherited through multiple generations, is a proposed goal of the application. However, sections of the specification, e.g. pp 4-5, bridging para. and pp 7-8, specifically state that one useful application of the current invention would be to remove DNA used for gene therapy from the germline so that it is **not** heritable.

Furthermore, deletion of DNA using the methods and nucleic acids as claimed could be utilized in cells other than ES cells (e.g. egg cells). Also, Applicants disagree with the Examiner's comments that the generation of transgenic organisms in species other than the

mouse is unpredictable. Transgenic organisms can be generated in many other organisms other than the mouse. As evidence of the predictability of transgenic organisms, Applicants submit the existence of the following references, abstracts of which are submitted herewith: "Transgenesis in nonmurine species," Mullins and Mullins, *Hypertension* 22(4):630-3 (1993); "Overexpression of hepatic lipase in transgenic rabbits leads to a marked reduction of plasma high density lipoproteins and intermediate density lipoproteins," Fan et al., *PNAS USA* 91(18):8724-8 (1994); "Estrogen augments the contribution of nitric oxide to blood pressure regulation in transgenic hypertensive rats expressing the mouse Ren-2 gene," Brosnihan et al., *Am.J. Hypertens.* 7(7 Pt 1):576-82 (1994); "Transgenic production of a variant of human tissue-type plasminogen activator in goat milk: generation of transgenic goats and analysis of expression," Ebert et al., *Biotechnology* 9(9):835-8 (1991); "Generation of transgenic dairy cattle from transgene-analyzed and sexed embryos produced in vitro," Hyttinen et al. *Biotechnology* 12(6):606-8; "Development of transgenic sheep that express the visna virus envelope gene," Clements et al., *Virology* 200(2):370-80; "Recombinant hemoglobin A produced in transgenic swine: structural equivalence with human hemoglobin A," Rao et al., *Artif. Cells Blood Substit. Imobil. Biotechnol.* 22(3):695-700.

In view of the amendments to the claims and above arguments, it is believed all of the claims are in condition for allowance, and Applicants request that the rejection of the claims under 35 U.S.C. § 112, first paragraph for lack of enablement be withdrawn.

35 U.S.C. 112, Ssecond Paragraph Rejections

The Examiner has also rejected claim 20 and the claims that depend therefrom under 35 U.S.C. 112, second paragraph, for being unclear for reciting components but not the order in which the components can be assembled. Claim 20 has been amended to recite the claimed components in sequential order.

In view of the amendments to the claims and above arguments, it is believed all of the claims are in condition for allowance and Applicants request that the rejection of the claims under 35 U.S.C. § 112, second paragraph, for being unclear be withdrawn.

In view of the above amendments and remarks, it is believed that all of the claims of the present application satisfy the provisions of the patent statutes. Reconsideration of this application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it will help expedite the allowance of the application.

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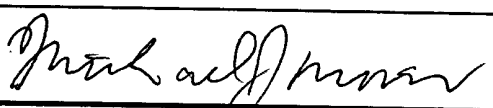
<tr><td>SIGNATURE</td><td colspan="2">  </td><td>DATE</td><td colspan="2">26 February 2003</td></tr>
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139A-II.AM4

1 : *Hypertension* 1993 Oct;22(4):630-3

Transgenesis in nonmurine species.

Mullins JJ, Mullins LJ

AFRC Centre for Genome Research, University of Edinburgh, UK.

Although the mouse remains the species of choice for most transgenic experimentation, it may be preferable or even necessary to use alternative species for certain applications. We review the strategies by which transgenic technology has been applied to other animals, specifically, the rat, rabbit, pig, sheep, goat, and cow. Additionally, we outline the potential applications of alternative transgenic species with reference to the field of hypertension and cardiovascular research.

Publication Types:
- Review
- Review, tutorial

PMID: 8406669, UI: 94011150

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1 : *Proc Natl Acad Sci U S A* 1994 Aug 30;91(18):8724-8

Overexpression of hepatic lipase in transgenic rabbits leads to a marked reduction of plasma high density lipoproteins and intermediate density lipoproteins.

Fan J, Wang J, Bensadoun A, Lauer SJ, Dang Q, Mahley RW, Taylor JM

Gladstone Institute of Cardiovascular Disease, Cardiovascular Research Institute, San Francisco, CA 94141.

To elucidate the precise metabolic roles of hepatic lipase (HL), a human HL cDNA in a liver-specific expression vector was used to generate transgenic lines in the rabbit, an animal that normally expresses low levels of this enzyme. HL was detected in the plasma of all rabbits only after the administration of heparin; HL activity in transgenic rabbits was found at levels up to 80-fold greater than that in nontransgenic littermates. This increase in enzyme activity was associated with as much as a 5-fold decrease in total plasma cholesterol levels. Expression of the transgene resulted in a dramatic reduction in the level of large high density lipoproteins (HDL1 and HDL2) as well as dense HDL3. A reduction in the quantity of intermediate density lipoproteins (IDL) was also observed. These results demonstrate that HL functions in the metabolism of HDL and IDL, thereby playing a key role in plasma cholesterol homeostasis.

PMID: 8078949, UI: 94360004

1 : *Am J Hypertens* 1994 Jul;7(7 Pt 1):576-82

Estrogen augments the contribution of nitric oxide to blood pressure regulation in transgenic hypertensive rats expressing the mouse Ren-2 gene.

Brosnihan KB, Moriguchi A, Nakamoto H, Dean RH, Ganten D, Ferrario CM

Hypertension Center, Bowman Gray School of Medicine of Wake Forest University, Winston Salem, North Carolina 27157.

Transgenic rats carrying the mouse Ren-2 gene (Ren-2d)27 provide a unique model to study the interplay between the renin-angiotensin system and estrogen in the pathogenesis of hypertension. In this study we measured the effects of ovariectomy and estrogen replacement on blood pressure and the contribution of vascular endothelium relaxing factor, nitric oxide, in female transgenic hypertensive rats and normotensive Sprague-Dawley (SD) rats. Both groups of animals were either ovariectomized or sham-operated at 12 weeks of age. Ovariectomized rats were treated with either 17 beta-estradiol (70 micrograms/day) or placebo for 4 weeks, whereas sham-operated rats received placebo alone. Mean arterial blood pressure measured in conscious rats directly by an arterial catheter was significantly higher in ovariectomized rats, compared with ovariectomized rats given estrogen replacement therapy for both transgenic (167 +/- 5 v 154 +/- 4 mm Hg, $P < .05$) and SD rats (125 +/- 4 v 113 +/- 5 mm Hg, $P < .05$). The contribution of endothelium-derived nitric oxide to the maintenance of blood pressure was examined by acute systemic injection of NG-monomethyl-L-arginine (L-NMMA, 10 mg/kg). L-NMMA caused a significantly greater increase in blood pressure in sham-operated transgenic as compared to SD rats (34 +/- 3 v 14 +/- 3 mm Hg, $P < .05$). The response in ovariectomized transgenic rats was markedly reduced (13 +/- 3 mm Hg), reaching levels that were no different from sham-operated SD rats.

PMID: 7946157, UI: 95032979

1 : *Biotechnology (N Y)* 1991 Sep;9(9):835-8

Transgenic production of a variant of human tissue-type plasminogen activator in goat milk: generation of transgenic goats and analysis of expression.

Ebert KM, Selgrath JP, DiTullio P, Denman J, Smith TE, Memon MA, Schindler JE, Monastersky GM, Vitale JA, Gordon K

Tufts University School of Veterinary Medicine, North Grafton, MA 01536-1895.

We report the first successful production of transgenic goats that express a heterologous protein in their milk. The production of a glycosylation variant of human tPA (LAtPA--longer acting tissue plasminogen activator) from an expression vector containing the murine whey acid promoter (WAP) operatively linked to the cDNA of a modified version of human tPA was examined in transgenic dairy goats. Two transgenic goats were identified from 29 animals born. The first animal, a female, was mated and allowed to carry the pregnancy to term. Milk was obtained upon parturition and was shown to contain enzymatically active LAtPA at a concentration of 3 micrograms/ml.

PMID: 1367544, UI: 91354712

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1 : *Biotechnology (N Y)* 1994 Jun;12(6):606-8

Generation of transgenic dairy cattle from transgene-analyzed and sexed embryos produced in vitro.

Hyttinen JM, Peura T, Tolvanen M, Aalto J, Alhonen L, Sinervirta R, Halmekyto M, Myohanen S, Janne J

Department of Biochemistry & Biotechnology, A.I. Virtanen Institute, University of Kuopio, Finland.

We have generated a transgenic calf from in vitro produced bovine embryos which had undergone transgene analysis and sexing prior to the embryo transfer. Bovine oocytes were isolated from slaughter-house-derived ovaries, matured and fertilized in vitro and subsequently microinjected with a dam-methylated gene construct consisting of genomic sequences encoding human erythropoietin and governed by bovine alpha S1-casein regulatory sequences. After 6 to 7 days in culture, the embryos were biopsied and while the embryo remained in culture, the biopsy was subjected to transgene analysis and sexing. The transgene analysis was accomplished with a combined treatment of the embryo lysates with DpnI restriction endonuclease and Bal31 exonuclease followed by polymerase chain reaction (PCR). The transgene analysis was based on the fact that DpnI only cleaves its recognition sequence if the adenine in the sequence is methylated. Pregnancy was induced by the transfer of three viable female embryos with a distinct transgene signal to a hormonally synchronized heifer recipient. Amniotic fluid analysis performed two months after the embryo transfer confirmed the presence of the transgene. The calf born was found to be transgenic by PCR analysis from blood, ear and fetal membranes. The presence of the transgene was also confirmed by Southern blotting.

PMID: 7764950, UI: 94288976

1 : *Virology* 1994 May 1;200(2):370-80

Development of transgenic sheep that express the visna virus envelope gene.

Clements JE, Wall RJ, Narayan O, Hauer D, Schoborg R, Sheffer D, Powell A, Carruth LM, Zink MC, Rexroad CE

Johns Hopkins University School of Medicine, Department of Comparative Medicine, Baltimore, Maryland 21205.

The ovine lentiviruses cause encephalitis, pneumonia, and arthritis in sheep worldwide. Visna virus is a prototype of this family and the pathogenesis and molecular biology of the virus has been well characterized. The envelope proteins of visna virus are responsible for binding of virus to host cells and for causing cell fusion. The surface glycoprotein also elicits cellular and humoral immune responses to the virus, the former being thought to be responsible for eliminating infected cells as well as causing inflammatory lesions. In this study, transgenic sheep were constructed that expressed the envelope genes of visna virus under the control of the visna LTR to investigate the role of the env gene in the pathogenesis of lentiviral disease in its natural host. Three transgenic lambs were identified that contain the env transgene and express the envelope glycoproteins. These transgenic animals have remained healthy and expression of the viral gene has had no obvious deleterious effect. Expression of the visna envelope protein was demonstrated by cell fusion mediated by the envelope gene as well as by immunoprecipitation of the envelope proteins with monoclonal antibodies and immunofluorescence analyses of Env protein in cells. The target cell for visna virus replication in infected animals is the monocyte/macrophage. In natural infection, the level of viral gene expression in these cells increases with cell maturation. In the transgenic sheep, monocytes did not express the envelope glycoproteins until they differentiated into macrophages in vitro. Expression of the env mRNA in macrophages was quantitated by an RNase protection assay. In addition to expression in macrophages, the transgene was expressed by fibroblasts isolated from skin of the transgenic sheep. Expression of both the Env and Rev proteins was detected by immunoprecipitation and immunofluorescence. Two of the three lambs responded immunologically to the expression of the transgene by producing binding antibodies to the

envelope glycoproteins. Thus, these transgenic sheep provide a model to study whether a lentivirus glycoprotein will prevent infection or modulate disease in its natural host after virus challenge.

PMID: 8178428, UI: 94233701

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1 : *Artif Cells Blood Substit Immobil Biotechnol* 1994;22(3):695-700

Recombinant hemoglobin A produced in transgenic swine: structural equivalence with human hemoglobin A.

Rao MJ, Schneider K, Chait BT, Chao TL, Keller H, Anderson S, Manjula BN, Kumar R, Acharya AS

Division of Hematology, Albert Einstein College of Medicine, Bronx, NY 10461.

Recombinant human hemoglobin A produced by coexpressing human alpha and beta globin genes in swine, and purified from the lysate of transgenic swine has been subjected to detailed protein chemical analysis. These structural studies involving laser desorption mass spectrometry, separation of globin chains by RPHPLC, amino terminal sequence analysis of the isolated globin chains, the tryptic peptide mapping of the purified globin chains and the amino acid composition analysis of the purified tryptic peptides of globin chains have established the primary structural equivalence of the globin chains of the transgenic swine derived hemoglobin A with that of human hemoglobin A. These results demonstrate that the transgenic swine system correctly translates the human alpha and beta globin m-RNA; carries out the correct cotranslational processing of globin chains, and does not introduce any unwanted post translational modifications into the mature chains. Thus, the transgenic swine expression system is an excellent approach for the production of HbA for developing an effective hemoglobin based oxygen carrier.

PMID: 7994390, UI: 95086643